ELSEVIER

Biochemical Pharmacology 63 (2002) 1807–1815

Biochemical Pharmacology

Formation and longevity of idarubicin-induced DNA topoisomerase II cleavable complexes in K562 human leukaemia cells

Elaine Willmore[a], Fiona Errington[a], Michael J. Tilby[b], Caroline A. Austin[a,*]

[a]*School of Biochemistry & Genetics, Medical School, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE2 4HH, UK*
[b]*The Cancer Research Unit, Medical School, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE2 4HH, UK*

Received 26 November 2001; accepted 26 February 2002

Abstract

Idarubicin (IDA) is an anthracycline used during treatment of acute myelogenous leukaemia (AML) and is clinically important because of its potency and lipophilicity (compared to the related compounds daunorubicin and doxorubicin). These drugs target DNA topoisomerase II (topo II), a nuclear enzyme that regulates DNA topology. Topo II poisoning leads to the trapping of an intermediate in the enzyme's cycle termed the ''cleavable complex.'' This study aims to increase understanding of drug interactions by use of the 'TARDIS' (trapped in agarose DNA immunostaining) assay to measure drug-induced topo II cleavable complexes in individual cells treated with anthracyclines. Mammalian cells contain two isoforms of topo II (α and β) and the TARDIS assay enables visualisation of isoform-specific complexes. Drug-treated cells were embedded in agarose, lysed and incubated with anti-topo II antibodies to microscopically detect topo IIα or β complexes. Results for K562 cells (at clinically relevant concentrations) showed that IDA and idarubicinol, its metabolite, formed mainly topo IIα cleavable complexes, the level of which decreases at doses >1 μM for IDA. Our data suggest that this decrease is due to catalytic inhibition by IDA at these doses. Doxorubicin formed low levels of topo IIα complexes and negligible topo IIβ complexes. In cytotoxicity studies, IDA and idarubicinol were equipotent, but both were more potent than daunorubicin and doxorubicin. We showed for the first time that there was a persistent increase in levels of topo IIα cleavable complexes after removal of IDA, suggesting that its greater effectiveness may be associated with both the longevity and high levels of these complexes.

Keywords: DNA topoisomerase II; TARDIS; Cleavable complex; Idarubicin; Leukaemia; K562 cells

1. Introduction

IDA is increasingly the anthracycline of choice for the treatment of AML and it forms the basis of several worldwide trials for induction therapy for AML [1]. Other important chemotherapeutic agents in this family of drugs include doxorubicin (DOX) (adriamycin, used in the treatment of solid tumours) and daunorubicin (DAUN) (also used in AML) (reviewed in [2]). There is much current clinical interest in IDA, because of its increased potency and lower cardiotoxicity. Additionally, its oral formulation makes it ideally suited for 'out-patient' treatment of elderly patients with poor prognosis, thus improving their quality of life [3]. The major metabolite, idarubicinol (IDAol), is unique in retaining the high activity of the parent compound (reviewed in [4]) and the high level of cellular accumulation may also contribute to its efficacy [5]. IDA can help overcome multidrug resistance, a phenomenon that provides a continuing clinical challenge. Cells which are resistant to other anthracyclines retain sensitivity to IDA, because the markedly high uptake in cells [6]. This means that the rate of drug efflux by P-glycoprotein cannot match the high rate of transmembrane transport [7,8]. Additionally, the high affinity of IDA for P-glycoprotein may result in its slow efflux from the cell [9].

It is now accepted that a major target of these agents is topo II (EC 5.99.1.3) [10], although other cytotoxic mechanisms (e.g. formation of free oxygen radicals, inhibition of DNA helicases, apoptosis and direct membrane effects) have been ascribed (reviewed in [11]). Mammals have two isoforms of topo II (α, 170 kDa and β, 180 kDa), and both are able to modulate DNA topology (reviewed in [12]). The anthracyclines inhibit topo II *via* the formation of drug-stabilised topo II cleavable complexes [13]. During

* Corresponding author. Tel.: +44-191-222-5251; fax: +44-191-222-7424.
E-mail address: caroline.austin@ncl.ac.uk (C.A. Austin).

Abbreviations: AML, acute myeloid leukaemia; topo II, DNA topoisomerase II; IDA, idarubicin; IDAol, idarubicinol; DAUN, daunorubicin; DOX, doxorubicin; ara-C, cytosine arabinoside.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)00920-6

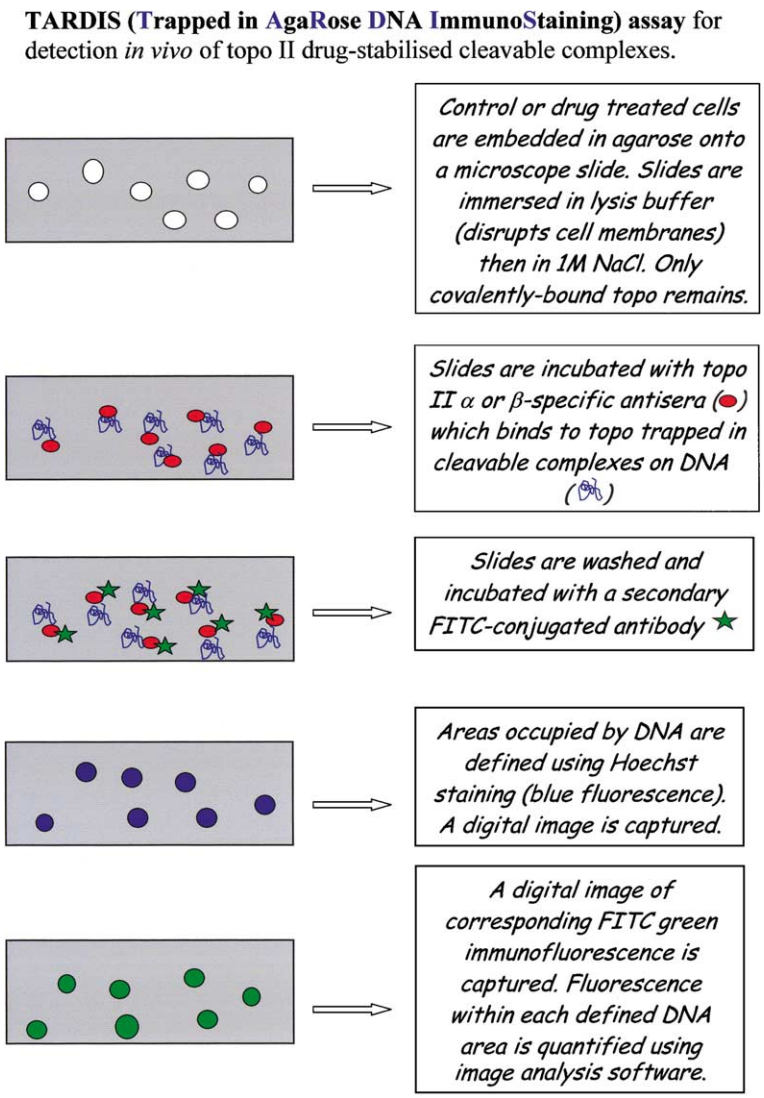

Fig. 1. TARDIS method.

the normal catalytic cycle of the enzyme, cleavable complexes are transient enzyme-bridged DNA double strand breaks which allow topo II to untangle DNA and facilitate cellular processes such as replication, transcription, recombination and chromatin remodelling. Upon drug treatment these complexes become stabilised, and cellular processing changes the protein-bridged break into a permanent strand break that ultimately leads to cell death (reviewed in [14]).

The aim of this study is to obtain further insights into the mechanism of action of IDA. Many clinical trials have demonstrated that IDA is preferable to DAUN (in terms of event-free survival) when used in conjunction with cytosine arabinoside (ara-C) for induction therapy for AML [1,15,16]. Some information regarding the molecular mechanisms of IDA is available, however one of the aspects that remains unclear is the isoform specificity of this agent in terms of cytotoxicity and cleavable complexes. We used the TARDIS assay (Fig. 1 and [17]) to quantify topo II cleavable complex formation and our results indicate that topo IIα is the target of IDA. Studies on the murine topo IIβ$^{-/-}$ knockout cell line (mtop2β-5) and a parental wild type topo IIβ$^{+/+}$ cell line (mTOP2β-4) confirmed that the β isoform is not the major cytotoxic target for IDA.

Growth inhibition assays demonstrated that IDA and IDAol were more potent than DAUN and DOX in the human leukaemia cell line K562, and this was paralleled by higher levels of topo IIα cleavable complexes compared to DAUN and DOX. Furthermore, IDA-induced complexes continued to rise after removal of drug from the cell culture medium and they remained for at least 48 hr. This persistence of cleavable complexes may explain the clinical efficacy of IDA.

2. Materials and methods

2.1. Cell culture

K562 is a cell line derived from a patient with chronic myelogenous leukaemia, but its metabolism is similar to

that of AML blasts [18] and therefore it is appropriate for studying mechanisms of action of anthracyclines in AML. K562 cells were maintained as a suspension culture in RPMI 1640 medium supplemented with 10% foetal bovine serum and penicillin (50 U/mL)/streptomycin (50 μg/mL). The murine topo IIβ$^{-/-}$ embryonic fibroblast cell line (mtop2β-5) and a wild-type topo IIβ$^{+/+}$ cell line (mTOP2β-4) were a gift from J. Wang (Harvard University, MS, USA) and were grown as monolayers in DMEM supplemented with 10% foetal bovine serum and penicillin/streptomycin [19,20]. All cell lines were maintained at 37° (5% CO_2) and were found to be consistently free of *mycoplasma* contamination. Cell culture reagents were obtained from Life Technologies.

2.2. Drugs

Solutions of drugs (1 mM in water, stored as small aliquots at −20°) were thawed immediately prior to use. The ara-C, DAUN and DOX were purchased from Sigma. IDA and IDAol were kindly provided by Pharmacia-UpJohn (UK).

2.3. Antibodies

Anti-topo II polyclonal antibodies were raised in rabbits. A total of 18511 was raised to recombinant human topo IIα and 18513 to a recombinant human topo IIβ C-terminal fragment. Western blots demonstrated that 18511 detected the α isoform specifically and 18513 detected the β isoform specifically [21]. In these assays, 18511(α) was used at a 1:50 dilution and 18513(β) at 1:200. The anti-rabbit FITC-conjugated second antibody ($F(ab')_2$ fragment, Sigma was used at 1:100 dilution).

2.4. Clonogenic assay

Murine topo IIβ$^{+/+}$ (mTOP2β-4) and topo IIβ$^{-/-}$ (mtop2β-5) cells were seeded (3×10^5 per plate) into 9 cm plates. After 48 hr, drug was added for 2 hr and the clonogenic assay was carried out as previously described [22].

2.5. Growth inhibition assay

Exponentially growing K562 cells (4×10^5/mL in six-well plates) were exposed to drug as described in the figure legends. They were then centrifuged, washed in PBS (0.14 M NaCl, 0.01 M phosphate, 0.002 M KCl) and resuspended in fresh medium. After 5 days, cells were counted by Trypan blue exclusion and the results expressed as described previously [23] as a percentage inhibition of growth compared to controls.

2.6. TARDIS assay

This has been described in detail previously [17] and is summarised in Fig. 1.

2.7. Quantitative fluorescence microscopy and image analysis

This has been described in detail previously [24]. The system used was a Leica DMLB microscope (Optivision) with Life Science Resources CCD digital camera and Pixel software (Life Science Resources). Statistical analysis was carried out using Graphpad Prism software (www.graphpad.com).

3. Results

3.1. Cytotoxicity studies

Table 1 shows the sensitivity of K562 cells to growth inhibition after a 2 hr exposure to the anthracyclines. As expected, IDA and IDAol were found to be the most potent, with an IC_{50} value of 0.02 μM. Fig. 2 shows for the first time the effect of anthracyclines on the survival of cells lacking topo IIβ. The IC_{50} values (Table 1) show the same order of potency of these agents as for the growth inhibition of K562 cells. There was no significant difference in survival between the mTOP2β-4 and mtop2β-5 cell lines for any of these drugs, suggesting that topo IIβ is not required for the cytotoxicity of these agents.

Table 1
The IC_{50} values for K562 (growth inhibition), mTOP2β-4 or mtop2β-5 cells (clonogenic survival) for several different agents tested in our laboratory, after a 2 hr exposure to drug

Compound	IC_{50}, K562 human leukaemia cells (μM)	IC_{50}, topo IIβ$^{+/+}$ (mTOP2β-4) (μM)	IC_{50}, topo IIβ$^{-/-}$ (mtop2β-5) (μM)	Statistical significance
Idarubicin	0.02	0.02	0.03	ns
Idarubicinol	0.02	0.02	0.02	ns
Daunorubicin	0.08	0.08	0.08	ns
Doxorubicin	0.2	0.36	0.28	ns
Etoposide	nt	1.7	2.1	ns
Mitoxantrone	nt	4.9	5.2	ns
mAMSA	nt	0.06	0.08	s, $P = 0.0014$

The IC_{50} values of mTOP2β-4 and mtop2β-5 cells were subjected to Student's *t*-test to compare cell kill between parent cells and cells lacking topo IIβ; ns: not significant (at the 5% level), s: significant with corresponding *P* value, nt: not tested.

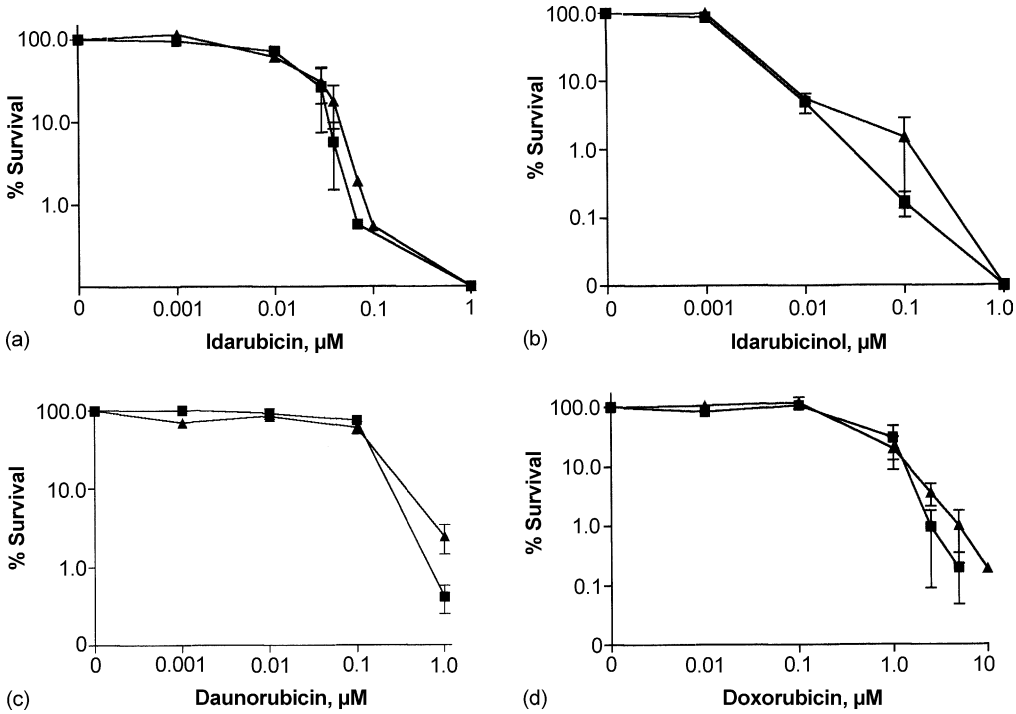

Fig. 2. Clonogenic survival curves for mTOP2β-4 cells (■) and mtop2β-5 cells (▲) after a 2 hr exposure to (a) IDA; (b) IDAol; (c) DAUN; or (d) DOX. Each point is the mean of at least three independent experiments with standard error bars shown.

3.2. Immunofluorescent staining of cleavable complexes using the TARDIS assay

K562 cells were exposed to a range of concentrations of IDA for 2 hr and the TARDIS assay ([17], summarised in Fig. 1) was used to detect drug-stabilised topo IIα and β cleavable complexes in individual cells from these cultures. Fig. 3 shows five pairs of images typical of those seen after staining with the 18511(α) antibody. Blue Hoechst-staining shows the location of the DNA whilst the corresponding green immunofluorescence was associated with topo IIα cleavable complexes. There was no green immunofluorescence associated with the DNA from untreated cells, but there was a dose-dependent increase in immunofluorescence in cells treated with 0.01, 0.1 or 1 μM IDA. At 10 μM IDA, however, the immunofluorescence decreased.

3.3. Quantification of immunofluorescent staining of cleavable complexes

Fig. 4 shows cleavable complex levels (after quantification) in K562 cells that had been treated with anthracyclines. Fig. 4(a) shows data for topo IIα cleavable complexes in cells that were treated with IDA. There was a significant difference from untreated controls at 0.1 μM ($P = 0.0011$, Student's *t*-test), and the levels reached a maximum at 1 μM. At 10 μM, the levels decreased, and were significantly lower than at 1 μM ($P = 0.003$) but not significantly different from levels in the untreated control ($P = 0.149$). IDAol formed similar levels of topo IIα cleavable complexes to IDA, with a maximum at 1 μM, but unlike IDA, at 10 μM levels were not significantly different to those at 1 μM ($P = 0.193$, *t*-test). By contrast, DOX (Fig. 4) and DAUN (data not shown) showed low levels of cleavable complex formation.

Fig. 4(b) shows data for topo IIβ. The *t*-test demonstrated that there was no significant difference in topo IIβ complex levels between control and drug-treated cells (for all doses) for IDA, IDAol and DOX (or DAUN, data not shown). We can exclude the possibility that this lack of topo IIβ complexes is because the TARDIS assay cannot detect them, since previous data from our laboratory using these antibodies [17,20] confirmed dose-dependent increases in topo IIβ complexes resulting from treatment with agents such as etoposide and mAMSA.

This data indicates firstly that levels of DOX-induced cleavable complexes are very low compared to those of IDA, and, secondly that although IDA does form high levels of cleavable complexes, these are mainly with topo IIα and not topo IIβ. It should be noted that IDA treatment did not lead to the formation of detectable levels of DNA topoisomerase I cleavable complexes (data not shown).

3.4. Cleavable complex stability

To investigate the longevity of topo IIα cleavable complexes after removal of IDA, K562 cells were exposed to 0.1 or 1 μM IDA for 2 hr, and then incubated in drug-free medium for various times before slide preparation. The results (Fig. 5) showed that levels of complexes showed no significant reduction (unlike results previously observed

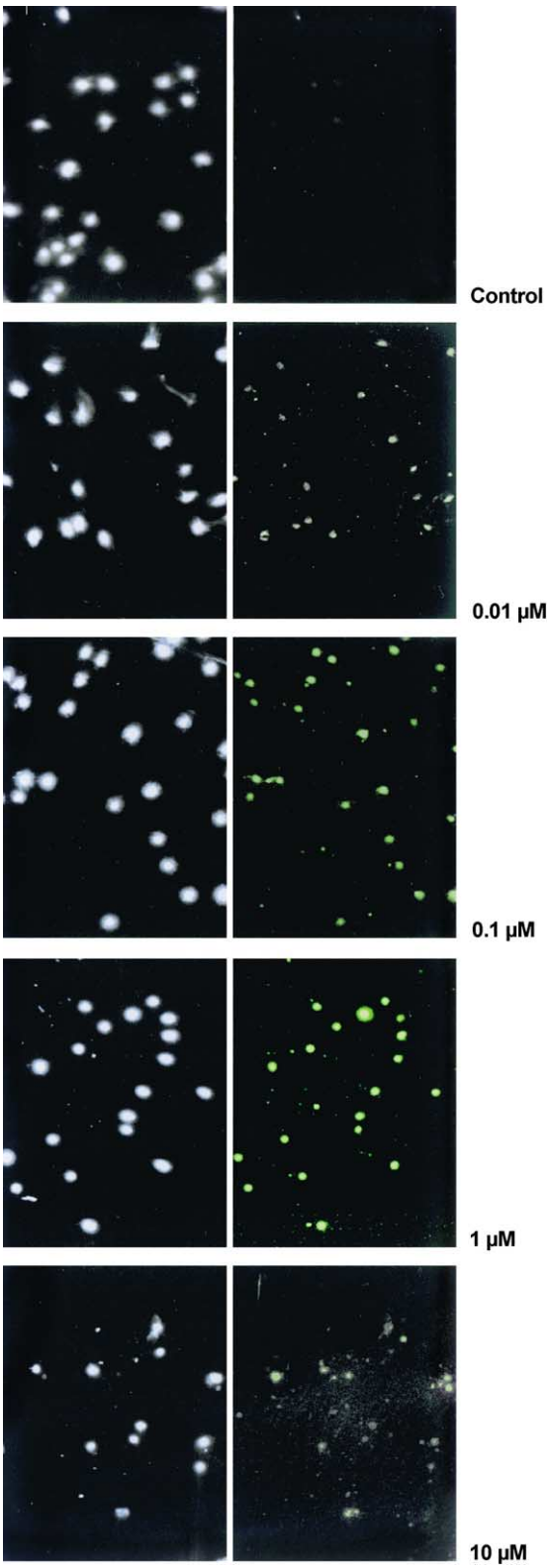

Fig. 3. Fluorescence of IDA-treated K562 cells. Cells were treated with IDA for 2 hr (at concentrations shown) before embedding and staining with 18511(α). Images on the left show the Hoechst-stained DNA (*blue*) whereas those on the right show corresponding FITC-stained (*green*) immunofluorescence. These images were captured under 10× objective and are typical of those seen in replicate experiments.

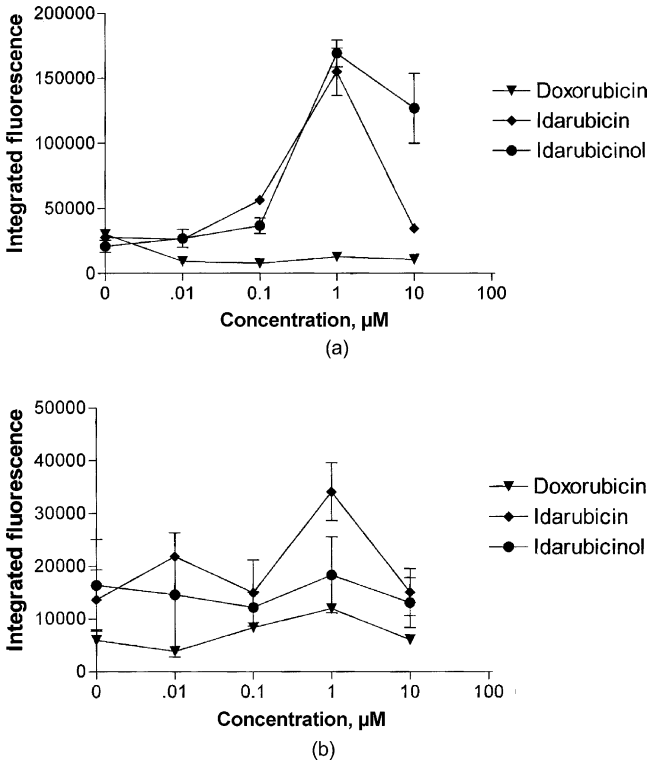

Fig. 4. Immunofluorescence levels in K562 cells after treatment with IDA (◆), IDAol (●) or DOX (▼). Cells were treated with 0, 0.01, 0.1, 1, or 10 μM drug for 2 hr, and then slides were prepared and stained with either (a) 18511(α) or (b) 18513(β). These were processed and analysed as described in the text. Plots show mean immunofluorescence values (with standard error bars) from four independent experiments (IDA, IDAol) or one experiment (DOX).

for etoposide [17]) rather, with increasing times after drug removal, the level of cleavable complexes dramatically increased.

Fig. 5 shows that after 5 hr incubation in drug-free medium, there was a significant increase in the amount of cleavable complexes (at the 1 μM dose, *t*-test, $P = 0.012$) compared with the same dose at time zero. In addition, increasing the length of time after drug removal significantly increased the level of cleavable complexes compared to time zero (at the 1 μM dose $P = 0.0026$ and 0.03 for 24 and 48 hr, respectively). Increasing the length of time in drug-free medium to more than 24 hr did not further increase or decrease the level of complexes (there was no significant difference at 1 μM between 24 and 48 hr). For the cells treated with 0.1 μM IDA, there was also an increase in cleavable complexes after drug removal, peaking at 48 hr. As expected, the level of cleavable complexes did not change with time for untreated cells.

3.5. Catalytic inhibition by IDA

The reduction in topo IIα cleavable complexes at 10 μM (a concentration that resulted in a high level of cytotoxicity) seemed paradoxical. It has been previously reported

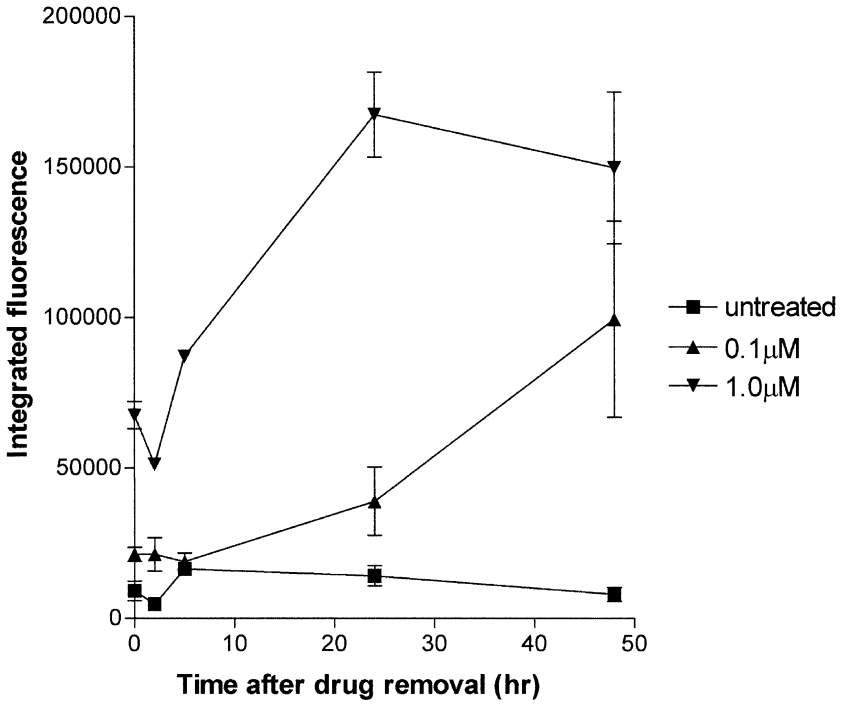

Fig. 5. Increase in topo II cleavable complexes with time. K562 cells were treated with IDA (0 (■), 0.1 (▲) or 1 μM (▼) for 2 hr, then slides were prepared either immediately, or after drug was washed out and cells were replaced in fresh medium for 2, 5, 24, 48 or 72 hr. Slides were stained with 18511(α) and processed using system I as described in the text. Plots show means (with standard error bars) for immunofluorescence values obtained from four independent experiments.

that "catalytic inhibitors" of topo II (such as the bisdioxopiperazines, e.g. ICRF-193 [25]) prevent stabilisation of cleavable complexes in cells exposed to topo II "poisons" (such as etoposide). This is because catalytic inhibitors block topo II from completing its reaction cycle. In the case of ICRF-193, this is due to trapping the enzyme in the closed clamp stage of the catalytic cycle [26], thereby preventing topo II poisons from forming cleavable complexes. In the case of aclarubicin (an anthracycline), catalytic inhibition results from the intercalation of the drug thus preventing topo II from binding to DNA [27]. In order to test the hypothesis that IDA might be behaving as a catalytic inhibitor at the higher dose, we used the established method [25,27] of testing whether a proposed topo II inhibitor can reduce cleavable complex formation by a topo II poison.

K562 cells were incubated with various concentrations of IDA for 2 hr, then either (a) the drug was washed off and cells placed in fresh medium containing 100 μM etoposide (IDA > E) or (b) 100 μM etoposide was added to the cells (without washing off IDA, IDA + E). In addition, cells were incubated with etoposide alone (2 hr) or IDA alone (for 2 or 4 hr—there was no significant difference). Fig. 6 shows that incubating cells with 1 μM IDA prior to etoposide treatment increased the level of etoposide-induced topo IIα cleavable complexes (compared to 100 μM etoposide alone, although this increase was not significant, $P = 0.27$, t-test). However, preincubation with 10 μM IDA significantly decreased the etoposide-induced

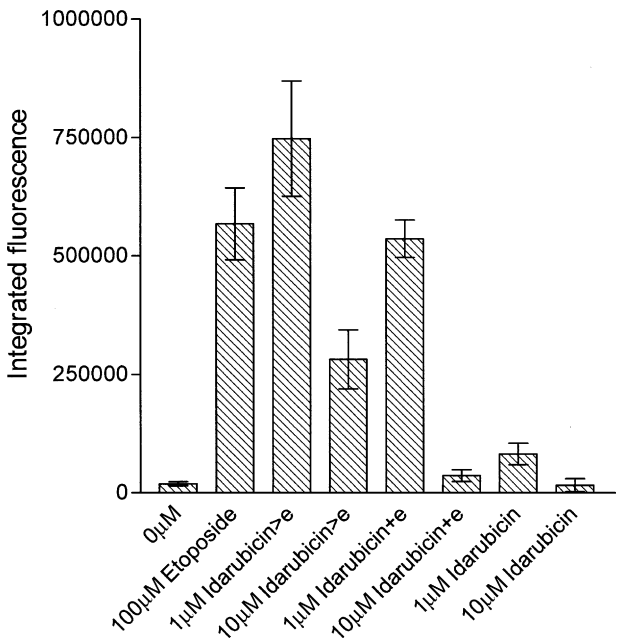

Fig. 6. Catalytic inhibition by IDA. K562 cells were treated with one of the following drug sequences. Cells were incubated with either 1 or 10 μM IDA for 2 hr, then IDA was washed off and cells were placed in fresh media containing 100 μM etoposide for 2 hr (IDA > E). Alternatively, cells were incubated with IDA for 2 hr, then etoposide was added directly into that medium for 2 hr (IDA + E). Cells were also treated with etoposide alone (2 hr, 100 μM) and IDA alone (2 hr, 1 and 10 μM) for comparison. Slides were prepared and stained with 18511(α), then processed using system I as described in the text. Results show the mean from three to five independent experiments with standard error bars.

complexes (compared to etoposide alone, $P = 0.032$). When cells were incubated with 1 μM IDA prior to and concurrently with etoposide, there was no significant difference in levels of etoposide-induced cleavable complexes, but preincubation with 10 μM IDA completely inhibited complex formation (highly significant, $P = 0.0004$). These results support the hypothesis that IDA is acting as a catalytic inhibitor of topo IIα at the 10 μM concentration.

4. Discussion

We have shown that in K562 and mouse embryonic fibroblast cells IDAol retained the cytotoxicity of the parent compound. These findings are consistent with those of Gieseler *et al*. [28], and also Kuffel *et al*. [29], who demonstrated that IDAol was equipotent to IDA in K562 cells, whereas other anthracycline C-13 metabolites (e.g. daunorubicinol and doxorubicinol) were up to 100-fold less potent than IDAol. Other data has shown IDA to be more potent than IDAol (e.g. [30]), but these differences may be cell line- and/or protocol-dependent.

In mammals, IDA is rapidly converted to its C-13-dihydro metabolite (IDAol) in the liver by the enzyme aldoketoreductase. The long half-life of IDAol (i.v. 63 hr, oral 46 hr [31]) is thought to contribute to the effectiveness of IDA in the clinic. The observation that both IDA and IDAol were more potent than DAUN (4-fold) and DOX (10-fold) is consistent with data from Binaschi *et al*. [32], who showed IDA to be 10-fold more potent than DAUN and 40-fold more potent than DOX in HL60 cells.

In order to determine the importance of topo IIβ in anthracycline-induced cytotoxicity, we used clonogenic assays on topo $IIβ^{-/-}$ and topo $IIβ^{+/+}$ cell lines (derived from the knockout mouse model [19]). This allows direct comparison of the effect of topo IIβ in mediating the cytotoxic response to these agents. The absence of topo IIβ did not affect the sensitivity of the IDA, IDAol, DAUN or DOX since there was no significant difference in IC_{50} values between the mTOP2β-4 and mtop2β-5 cell lines for each of these agents. This shows that topo IIβ is not necessary for the cytotoxic effects of IDA, IDAol, DAUN or DOX, supporting results from the TARDIS assay, where there were little or no topo IIβ cleavable complexes detected. By contrast, previous work from our laboratory using this model showed topo IIβ is an important target for acridines such as mAMSA, AMCA and mAMCA [20]. This illustrates the fact that responses of mammalian cells to topo II poisons can fall into distinct groups with respect to isoform specificity.

Our results suggest that topo IIα is targeted by the anthracyclines. This corroborates data obtained in a yeast model system [33], where DOX produced higher levels of cell killing in yeast expressing plasmid-borne human topo IIα than in those expressing human topo IIβ. Additionally, in similar experiments, yeast expressing human topo IIα were sensitive to IDA and DOX, showing that both agents target topo IIα [10]. The results in Fig. 4 showed that cleavable complexes became detectable at 0.1 μM, a highly cytotoxic concentration. It is possible that complexes are relatively low in abundance, and thus higher levels of IDA are required to detect complexes by this immunological technique, similar to the ICE bioassay [34]. It is not possible to quantify levels of cytotoxicity as a function of the number of complexes, since, for example, low levels of complexes on a particularly sensitive sequence of the genome could be highly cytotoxic. It is now accepted that the main mechanism by which topo II-targeting poisons exert their cytotoxicity is *via* formation of drug-stabilised cleavable complexes, and the resulting events which occur downstream of complex formation, e.g. DNA strand breakage [35]. We have shown that IDA and IDAol form higher levels of topo IIα drug-stabilised complexes than DAUN and DOX, in parallel to the order of potency of these agents.

It is clear that the sequence of DNA cleaved by topo II (in the absence or presence of drugs) is a determinant for cytotoxicity [36]. In addition to the level and location of cleavable complexes, their stability is also likely to be important for cytotoxicity. The longevity of cleavable complexes after IDA treatment, and the increase in complexes after drug removal (Fig. 5) is striking. There are several possible explanations for this phenomenon. Firstly, the DNA binding and affinity of IDA is higher compared to DAUN and DOX. In patient CML blasts, DAUN dissociates from the DNA within 60 min whereas IDA does not [28]. It is possible that IDA already bound to DNA may continue to form new cleavable complexes even after washing off unbound drug. Secondly, increased cellular retention [31] may allow IDA to continue to fill new DNA binding sites, thus allowing further interaction with topo II and further complex formation. Additionally, IDA is less susceptible to efflux *via* P-glycoprotein [37], and, even in resistant cells not expressing P-glycoprotein, IDA retains its cytotoxic potency more than DAUN.

Finally, other groups have studied protein-associated DNA strand breaks detected by alkaline elution (i.e. resulting from drug-induced topo II cleavable complexes). An increase in strand breaks after drug removal (analagous to the data in Fig. 5) has been reported for DOX [38,39] and the persistence of IDA-induced DNA breaks has been demonstrated [32,40]. These data, together with our data on IDA-induced complexes, demonstrate a property of anthracyclines that is contrary to that seen with other topo II drugs, i.e. the ability to induce persistent complexes that continue to increase DNA damage after drug removal.

The decline in cleavable complex levels at higher concentrations (Fig. 5) of IDA is consistent with observations on DNA strand breaks levels after IDA treatment (at similar concentrations) in P388 leukaemia cells [41]. This was attributed to inhibition of topo II binding (and, therefore catalytic activity) at higher doses of IDA, and it is

possible that such high concentrations lead to perturbations in transport of both IDA and etoposide. Other hypotheses suggest that there may be a structural change in DNA conformation [42] due to saturation of intercalator binding at higher doses. It is interesting that data in Fig. 6 suggests that IDA has a dual capacity as a poison and a catalytic inhibitor, depending upon the concentration used, and this has implications for the clinical administration of these drugs. Also, the increase in etoposide-induced complexes by preincubation with lower concentrations of IDA (Fig. 6) implies that this schedule may be better than concurrent incubation. Work is in progress to study the cytotoxic effects of this and other schedules that include topo II-targeting agents.

Significantly, several trials have demonstrated that IDA is superior to DAUN when used in conjunction with cytosine ara-C for induction therapy for AML [1,15,16]. We propose that the efficacy of IDA both *in vitro* and *in vivo* is related to the longevity of IDA-induced topo II cleavable complexes, and the potency of the metabolite, IDAol.

Acknowledgment

This work was funded by the Leukaemia Research Fund, UK (Grant 9743 (to E.W.) and 9683 (to F.E.)) and the Wellcome Trust (Grant 056232).

References

[1] AML Collaborative Group, A systematic collaborative overview of randomised trials comparing idarubicin with daunorubicin (or other anthracyclines) as induction therapy for acute myeloid leukaemia. Br J Haem 1998;103:100–9.

[2] Hande KR. Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta 1998;1400:173–84.

[3] Jackson GH, Taylor PRA, Iqbal A, Galloway MJ, Turner G, Haynes A, Hamilton PJ, Russell N, Proctor SJ. The use of an oral chemotherapy (idarubicin and etoposide) in the treatment of acute myeloid leukaemia in the elderly: a report of toxicity and efficacy. Leukaemia 1997;11:1193–6.

[4] Borchmann K, Hubel K, Schnell R, Engert A. Idarubicin: a brief overview on pharmacology and clinical use. Int J Clin Pharmacol Ther 1997;35:80–3.

[5] Bogush T, Robert J. Comparative evaluation of the intracellular accumulation and DNA binding of idarubicin and daunorubicin in sensitive and multidrug-resistant human leukaemia K562 cells. Anticancer Res 1996;16:365–8.

[6] Manketkorn S, Dubru F, Hesschenbrouck J, Fiallo M, Garnier-Suillerot A. Relation among the resistance factor, kinetics of uptake, and kinetics of the P-glycoprotein-mediated efflux of doxorubicin, dainorubicin, 8-(S)-fluoroidarubicin and idarubicin in multidrug-resistant K562 cells. Mol Pharmacol 1996;49:532–9.

[7] Broxterman HJ, Sonneveld P, van Putten WJL, Lankelma J, Eekman CA, Ossenkoppele GJ, Pinedo HM, Lowenberg B, Schuurrhuis GJ. P-glycoprotein in primary acute myeloid leukaemia and treatment outcome of idarubicin/cytosine arabinoside-based induction therapy. Leukaemia 2000;14:1018–24.

[8] Marbeuf-Gueye C, Ettori D, Priebe W, Kozlowski H, Garnier-Suillerot A. Correlation between the kinetics of anthracycline uptake and the resistance factor in cancer cells expressing the multidrug resistance protein or the P-glycoprotein. Biochim Biophys Acta 1999;1450:374–84.

[9] Wielinga PR, Westerhoff HV, Lankelma J. The relative importance of passive and P-glycoprotein mediated anthracycline efflux from multidrug-resistant cells. Eur J Biochem 2000;267:649–57.

[10] Binaschi M, Farinosi R, Austin CA, Fisher LM, Zunino F, Capranico G. Human DNA topoisomerase IIα-dependent DNA cleavage and yeast cell killing by anthracycline analogues. Cancer Res 1998;58:1886–92.

[11] Gerwirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumour effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol 1999;57:727–41.

[12] Austin CA, Marsh KL. Eukaryotic DNA topoisomerase IIβ. Bioessays 1998;20:215–26.

[13] Zunino F, Capranico G. DNA topoisomerase II as the primary target of antitumour anthracyclines. Anticancer Drug Design 1990;5:307–17.

[14] Fortune JM, Osheroff N. Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice. Prog Nucl Acid Res Mol Biol 2000;64:221–53.

[15] Reiffers J, Huguet F, Stoppa AM, Molina L, Marit G, Attal M, Gastaut JA, Michallet M, Lepeu G, Broustet A, Pris J, Maraninchi O, Hollard D, Faberes C, Mercier M, Hurteloup P, Danel P, Tellier Z, Berthaud P. A prospective randomized trial of idarubicin vs. daunorubicin in combination chemotherapy for acute myelogenous leukaemia of the age group 55–75. Leukaemia 1996;10:389–95.

[16] Berman E, Heller G, Santorsa J, McKenzie S, Gee T, Kempin S, Gulati S, Andreeff M, Kolitz J, Gabrilove J, Reich L, Mayer K, Keefe D, Trainor K, Schluger A, Penenberg D, Raymond V, O'Reilly R, Jhanwar S, Young C, Clarkson B. Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukaemia. Blood 1991;77:1666–74.

[17] Willmore E, Frank AJ, Padget K, Tilby MJ, Austin CA. Etoposide targets topoisomerase IIα and IIβ in leukaemic cells: isoform-specific cleavable complexes visualised and quantified *in situ* by a novel immunofluorescence technique. Mol Pharmocol 1998;53:78–85.

[18] Gandhi V, Estey E, Keating MJ, Plunkett W. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukaemia during therapy. J Clin Oncol 1993;11:116–24.

[19] Yang X, Li W, Prescott ED, Burden SJ, Wang JC. DNA topoisomerase IIβ and neural development. Science 2000;287:131–4.

[20] Errington F, Willmore E, Tilby MJ, Li L, Li G, Li W, Baguley BC, Austin CA. Murine transgenic cells lacking DNA topoisomerase IIβ are resistant to acridines and mitoxantrone: analysis of cytotoxicity and cleavable complex formation. Mol Pharmacol 1999;56:1309–16.

[21] Cowell IG, Willmore E, Chalton DA, Marsh KL, Jazrawi E, Fisher LM, Austin CA. Nuclear distribution of human DNA topoisomerase IIβ: a nuclear targeting signal resides in the 116-residue C-terminal tail. Exp Cell Res 1998;243:232–40.

[22] Moses K, Harris AL, Durkacz BW. Synergistic enhancement of 6-thioguanine cytotoxicity by ADP-ribosyltransferase inhibitors. Cancer Res 1988;48:5650–4.

[23] Boulton S, Pemberton LC, Porteous JK, Curtin NJ, Griffin RJ, Golding BT, Durkacz BW. Potentiation of temozolamide-induced cytotoxicity: a comparative study of the biological effects of poly(ADP-ribose) polymerase inhibitors. Br J Cancer 1995;72:849–56.

[24] Frank AJ, Proctor SJ, Tilby MJ. Detection and quantification of melphalan-DNA adducts at the single cell level in hematopoietic tumour cells. Blood 1996;88:977–84.

[25] Ishida R, Iwai M, Hara A, Andoh T. The combination of different types of antitumour topoisomerase II inhibitors, ICRF-193 and VP-16, has synergistic and antagonistic effects on cell survival, depending on treatment schedule. Anticancer Res 1996;16:2735–40.

[26] Jensen LH, Nitiss KC, Rose A, Dong J, Zhou J, Hu T, Osheroff N, Jensen PB, Sehested M, Nitiss JL. A novel mechanism of cell killing by anti-topoisomerase II bisdioxopiperazines. J Biol Chem 2000; 275:2137–46.

[27] Sehested M, Jensen PB. Mapping of DNA topoisomerase II poisons (etoposide, clerocidin) and catalytic inhibitors (aclarubicin, ICRF-187) to four distinct steps in the topoisomerase II catalytic cycle. Biochem Pharm 1996;51:879–86.

[28] Gieseler F, Biersack H, Brieden T, Manderscheid J, Nussler V. Cytotoxicity of anthracyclines: correlation with cellular uptake, intracellular distribution and DNA binding. Ann Hematol 1994; 69:s13–7.

[29] Kuffel MJ, Reid JM, Ames MM. Anthracyclines and their C-13 metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture. Cancer Chemother Pharmacol 1992;30:51–7.

[30] Fukushima T, Yamashita T, Yoshio N, Misaki H, Yamauchi T, Imamura S, Urasaki Y, Ueda T. Effect of PSC 833 on the cytotoxicity of idarubicin and idarubicinol in multidrug-resistant K562 cells. Leukaemia Res 1999;23:37–42.

[31] Gillies HC, Herriott D, Liang R, Ohashi K, Harper PG. Pharmacokinetics of idarubicin (4-demethoxydaunorubicin; IMI-30; NSC 256439) following intravenous and oral administration in patients with advanced cancer. Br J Clin Pharmacol 1987;23:303–10.

[32] Binaschi M, Capranico G, Dal Bo L, Zunino F. Relationships between lethal effects and topoisomerase II-mediated double-stranded DNA breaks produced by anthracyclines with different sequence specificity. Mol Pharmacol 1997;51:1053–9.

[33] Meczes EL, Marsh KL, Fisher LM, Rogers MR, Austin CA. Complementation of temperature-sensitive topoisomerase II mutations in *Saccharomyces cerevisiae* by a human TOP2β construct allows the study of topoisomerase IIβ inhibitors in yeast. Cancer Chemother Pharmacol 1997;39:367–75.

[34] Subramanian D, Rosenstein BS, Muller MT. Ultraviolet-induced DNA damage stimulates topoisomerase I–DNA complex formation *in vivo*: possible relationship with DNA repair. Cancer Res 1998; 58:976–84.

[35] Kaufmann S. Cell death induced by topoisomerase-targeted drugs: more questions than answers. Biochim Biophys Acta 1998;1400: 195–211.

[36] Cornarotti M, Tinelli S, Willmore E, Zunino F, Fisher LM, AustinCapranico CAG. Drug sensitivity and sequence specificity of human recombinant DNA topoismerase IIα(p170) and IIβ(p180). Mol Pharmacol 1996;50:1463–71.

[37] Ross DD, Doyle LA, Yang W, Tong Y, Cornblatt B. Susceptibility of idarubicin, daunorubicin, and their C-13 alcohol metabolites to transport-mediated multidrug resistance. Biochem Pharmocol 1995; 50:1673–83.

[38] Capranico G, De Isabella P, Penco S, Tinelli S, Zunino F. Role of DNA breakage in cytotoxicity of doxorubicin, 9-deoxydoxorubicin, and 4-demethyl-6-deoxydoxorubicin in murine leukaemia P388 cells. Cancer Res 1989;49:2022–7.

[39] Zwelling LA, Michaels S, Erickson LC, Ungerleider MN, Nichols M, Kohn K. Protein-associated deoxyribonucleic acid strand breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4′-(9-acridinylamino)methanesulfon-*m*-anisidide and adriamycin. Biochemistry 1981;20:6553–63.

[40] Capranico G, Tinelli S, Zunino F. Formation, resealing and persistance of DNA breaks produced by 4-demethoxydaunorubicin in P388 leukaemia cells. Chemico-Biol Int 1989;72:113–23.

[41] Capranico G, Soranzo C, Zunino F. Single-strand DNA breaks induced by chromophore-modified anthracyclines in p388 leukaemia cells. Cancer Res 1986;46:5499–503.

[42] Tewey KM, Chen GL, Nelson EM, Liu LF. Intercalative antitumour drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J Biol Chem 1984;259:9182–7.